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Decreased CD11b expression on circulating polymorphonuclear leukocytes in patients with extensive plaque psoriasis

In psoriasis, polymorphonuclear leukocytes are consistently present in the early psoriatic lesion and in actively spreading plaques. CD11b, which is part of the β2-integrin receptor Mac-1, plays an important role in various biological functions of the polymorphonuclear leucocyte such as leukocyte adhesion to endothelium, extravasation, tissue migration and degranulation. In the present study we investigated the possibility of systemic differences in leukocyte CD11b-expression between patients with extensive plaque psoriasis and healthy volunteers.
Venous blood samples were obtained from 15 patients with extensive plaque psoriasis (Psoriasis Area and Severity Index greater than 10.0), and from 15, matched, healthy controls. Both unstimulated and *in vitro* leukotriene B_4-stimulated leukocytes were stained for CD11b, which was quantified using flow cytometry methods. A tendency towards decreased basal CD11b expression was observed on leukocytes from psoriatic patients compared to healthy subjects. After *in vitro* stimulation with leukotriene B_4 (LTB_4), the difference between psoriasis patients and controls increased further and was statistically significant. Patients with unstable psoriasis (increasing size of individual lesions and/or pinpoint papules around chronic plaques) proved to have even lower unstimulated and LTB_4-stimulated CD11b expression. No correlation was found between CD11b expression and severity of psoriasis using the PASI-score. Interestingly, the relative CD11b up-regulation (ratio $CD11b_{LTB4\text{-}stimulated}/CD11b_{unstimulated}$) was virtually the same in both groups. Therefore, the signalling pathway from leukotriene B_4-receptor binding up to CD11b expression on the leukocyte surface, was essentially normal in psoriasis. It is hypothesised that the decreased CD11b expression in psoriasis patients is caused by leukocyte compartmentalisation. *(Key words: blood, CD11b, flow cytometry, leukotriene B_4, polymorphonuclear leukocyte, psoriasis.)*

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Cutaneous inflammation is an important aspect of the psoriatic lesion. Already in pinpoint psoriatic lesions, in the peripheral zone of the margin of psoriatic plaques, and during early relapse following discontinuation of treatment, accumulation of T-lymphocytes, monocytes, mast cells and polymorphonuclear leukocytes (PMN) can be observed [1]. No substantial inflammatory changes have been seen in the symptomless skin distant from the psoriatic lesion, although some authors claim increased density of T-lymphocytes [2-4]. Until now, no consensus has been reached on which cells are first in invading the "pre-psoriatic skin".
The invasion by polymorphonuclear leukocytes (PMN) in psoriatic skin is an early feature. The epidermal accumulation of PMN adopts the psoriasis-specific configurations of spongiform pustules of Kogoj in the stratum Malpighi, and microabcesses of Munro as intracorneal accumulations. In pustular psoriasis, PMN accumulation is the dominating feature [5, 6]. In peripheral blood, functional and biochemical characterisation has revealed normal, decreased and increased expression of PMN activation features [7]. The activity of the psoriatic process whether expanding or stable proved to be an important factor for the characteristics of peripheral blood PMN [8].

Integrin adhesion molecules are of great importance in intercellular and cell-matrix interactions [9]. They consist of non-covalently linked α- and β-chains and are categorised by their β-chain [10]. The Mac-1-integrin (CD11b/CD18) which is part of the β2-subpopulation, is known to be of particular importance to various PMN functions [11-13]. It is pivotal to PMN adhesion to vascular endothelium, extravasation, tissue migration, the oxidative burst, and degranulation.
The Mac-1-receptor can be up-regulated by various biochemical compounds such as formyl-Met-Leu-Phe, platelet activating factor, interleukin 8, and leukotriene B_4 [14-17]. To the best of our knowledge, there are no known active down-modulators of Mac-1. In particular, the up-regulation of CD11b by leukotriene B_4 (LTB_4) is of interest since LTB_4 is produced in large quantities in psoriatic lesional skin [18-21]. LTB_4 which is formed in the arachidonic acid cascade, is a potent upregulator of CD11b. It causes a rapid increase in cell-surface presence through qualitative and quantitative up-regulation [11, 22-24]. We chose to evaluate CD11b as it is far more specific for PMN than CD18, which is also present on other leukocyte subsets.

In psoriatic skin, up to the most peripheral zone of the psoriatic lesion, the number of CD11b-positive cells is increased compared to normal skin [25], which suggests that the expression of CD11b by PMN is an important factor in the pathogenesis of psoriasis. So far, no information is available on CD11b expression by peripheral blood PMN of psoriatic patients.
A flow cytometrical study was performed in order to investigate whether there are any systemic changes detectable in the basal levels of PMN CD11b expression in patients with extensive plaque psoriasis compared to healthy volunteers. Secondly, we assessed whether PMN from psoriatics respond abnormally to *ex vivo* LTB_4-stimulation, with respect to CD11b-up-regulation.

Materials and methods

Subjects

Venous blood samples were obtained from 15 patients with extensive plaque psoriasis. All systemic antipsoriatic treatments were stopped at least three months prior to the investigation, and local antipsoriatic therapy was stopped for at least two weeks. Fifteen healthy volunteers, without any history or signs of skin disease, served as the control group. Subjects were at least 20 years of age. Both groups were matched for gender and age. No systemic, anti-inflammatory or immunomodulating drugs were allowed. The severity of plaque psoriasis was assessed by the psoriasis area and severity index (PASI). Patients were judged to have extensive plaque psoriasis when PASI was greater than 10.0.
Unstable plaque psoriasis was defined as an increasing size of the individual lesions during the two weeks preceding the study and/or the occurrence of pinpoint papules around chronic plaques. In stable psoriasis, these signs were not present.

CD11b integrin up-regulation assay [26]

Blood specimens for assessment of *ex vivo* neutrophil CD11b surface-expression were obtained. Peripheral blood (4 ml) was collected by venepuncture, kept in ethylene-di-amine-tetra-acetic-acid (EDTA) at 4° C and processed within 3 h of collection to prevent non-specific up-regulation of CD11b-expression as a result of neutrophil activation.
Blood samples were processed in triplicate using 90 µl aliquots which were incubated with LTB_4 (10 µl 1 x 10^{-7} M) in Hanks' balanced salt solution (Sigma Chemical Corp., St. Louis, USA) containing 0.1% bovine serum albumin (HBSS-BSA), or with HBSS (10 µl) alone for 30 min at 37° C. Samples were then cooled and incubated in the dark for 30 min at 4° C with 10 µl (0.045 g/l) anti-human CD11b-fluorescein conjugate (Mo-1-FITC, Coulter Corp., Hialeah, USA). Erythrocytes were lysed and the remaining cells were washed with HBSS-BSA, fixed in 1% paraformaldehyde solution and stored at 4° C until analysis.
The analysis was always performed within one week of preparation of the leukocyte suspensions, because previous experiments showed that assessment within 7 days minimises storage artefacts.

Flow cytometry analysis

All specimens were analysed on an Epics Elite Flow Cytometer (Coulter, Luton, UK). Cells were excited with an air-cooled 488 nm argon laser set at 15 mW. Green fluorescence (FITC) was measured through a 525 nm (band width 30 nm) band pass filter. Calibration and sensitivity were checked by using FITC-labelled beads (Standard-Brite, Coulter Source, Hialeah, USA). Forward and side scatter were used for gating granulocytes only. For each sample 5,000 gated cells were analysed *(Fig. 1)*.

Statistical analysis

For comparison between different groups, the Mann-Whitney test was used. Correlation between disease activity and CD11b-expression, and between CD11b-expression before and after *in vitro* LTB_4-stimulation was calculated using the Pearson test.

Results

Psoriatic patients (N = 15) and healthy controls (N = 15) were matched for gender and age. *Table I* summarizes the age of patients and healthy controls as well as the severity of psoriasis indicated by the PASI-score. Among the 15 patients with psoriasis, 8 patients had unstable psoriasis, characterised by aggravation of the lesions during the two weeks preceding the study and pinpoint papules around the chronic plaques.
The ages of patients and healthy controls were comparable. All patients were suffering from severe plaque psoriasis as indicated by the PASI-score. The extent of skin involvement did not differ between patients with unstable and stable psoriasis.

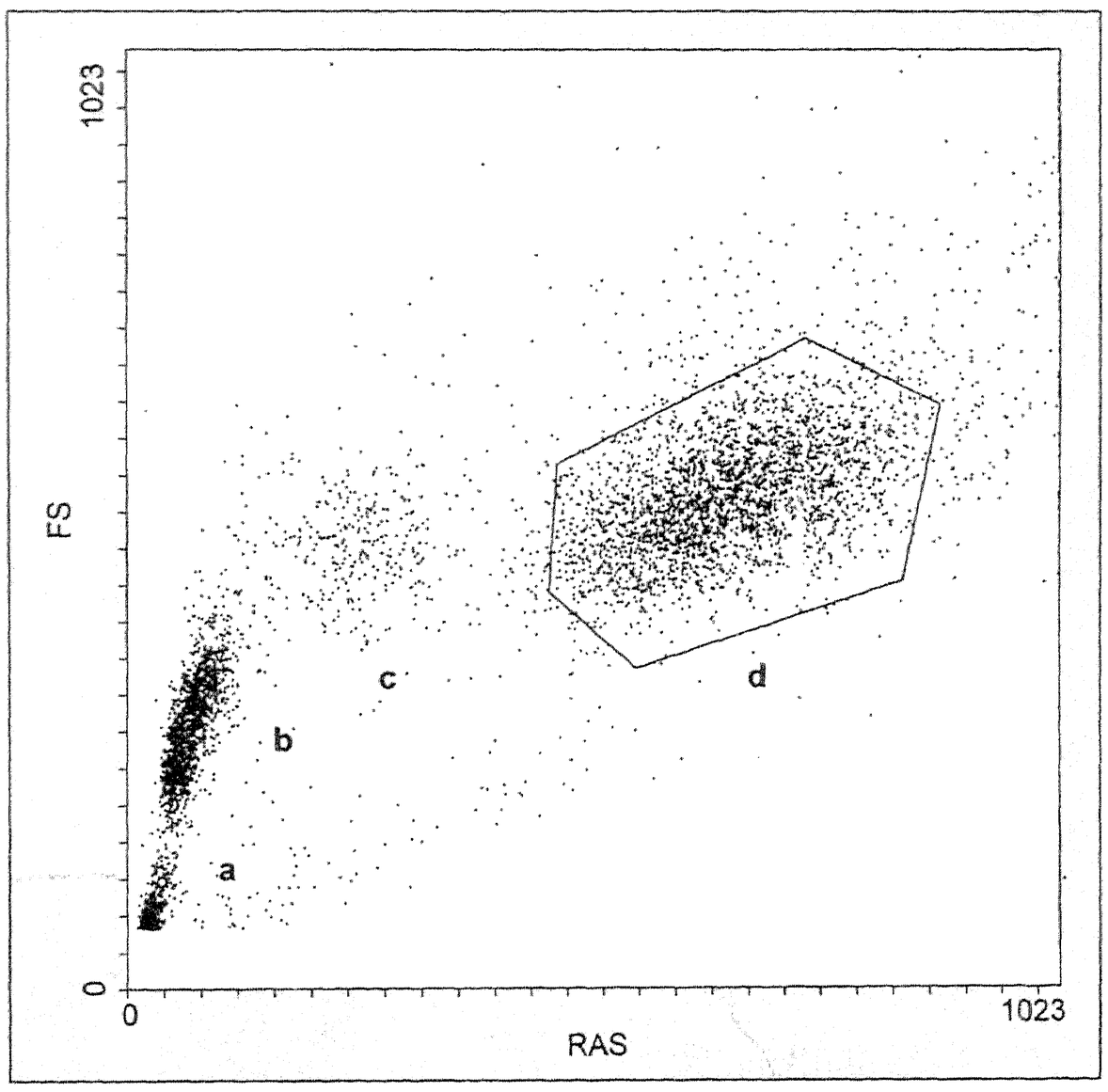

Figure 1. *Right angle scatter (RAS)* versus *forward scatter (FS) of a leukocyte suspension. Several distinct cell populations can be recognised: (a) unlysed erythrocytes and debris; (b) lymphocytes; (c) monocytes; (d) PMN. This population of PMN is gated out to assess the FITC-fluorescense of every cell which correlates with the number of CD11b molecules present on the cell surface.*

± 1.77 compared to 17.6 ± 1.13 in healthy volunteers. No relation was found between the individual severity of psoriasis as expressed by PASI, and PMN CD11b-expression.
In order to compensate for the interindividual variation of CD11b expression, the relative "CD11b up-regulatability" for every individual (PMN CD11b-expression after LTB_4-stimulation divided by CD11b-expression present on unstimulated volunteers. After *in vitro* stimulation of PMN by LTB_4 there was a clear decrease in PMN CD11b expression in psoriatic patients compared to healthy volunteers. No correlation was observed between the severity of psoriasis as expressed by the PASI-score, and PMN CD11b expression. Comparison of patients with unstable psoriasis and healthy subjects accentuated the difference between patients and healthy controls

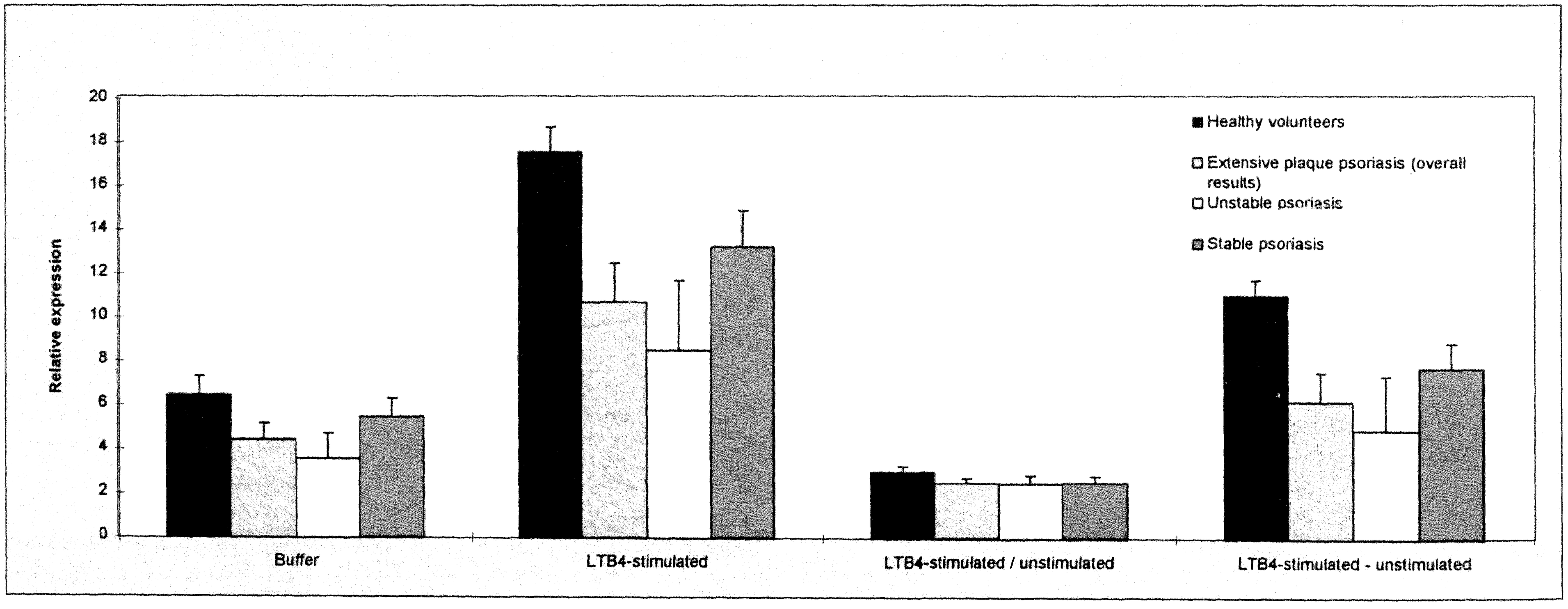

Figure 2. *CD11b surface-expression (mean fluorescence units per cell) of PMN in psoriatic patients and healthy volunteers.*

Table I. *Mean and standard error of the mean (SEM) of age, psoriasis area and severity index (PASI), and neutrophil CD11b-expression in the patient and volunteer group. Additionally, the psoriatic patients were divided in those who had stable and those who had unstable plaque psoriasis*

Patients	N	M/F	Years of age (mean ± SEM)	PASI (mean ± SEM)
Healthy controls	15	12/3	46.2 ± 3.8	not applicable
Psoriatic patients (overall group)	15	12/3	47.7 ± 2.9	17.9 ± 1.7
Patients with unstable psoriasis	8	6/2	50.6 ± 3.8	19.9 ± 2.9
Patients with stable psoriasis	7	6/1	44.4 ± 4.3	15.6 ± 1.0

The mean PMN CD11b expression, as measured by flow cytometry in psoriatic patients and healthy volunteers, is summarized in *Figure 2*. The basal CD11b expression in the group of psoriatic patients was 4.48 ± 0.72 (mean fluorescence units per cell ± SEM) and in healthy volunteers 6.50 ± 0.84 (mean fluorescence units per cell ± SEM). The difference between psoriatic and normal, unstimulated CD11b expression was not significant ($p = 0.06$), However, there seemed to be a tendency towards a decreased CD11b expression in psoriatics compared to controls.

The PMN CD11b-expression after *in vitro* stimulation with LTB_4 showed a highly significant difference between the patient and control group ($p = 0.001$). In the patient group, the presence of CD11b on the cell surface of PMN was 10.7 PMN) was calculated. Remarkably, this relative PMN CD11b up-regulation in psoriatic patients did not differ from that found in healthy volunteers (2.51 ± 0.23 and 3.03 ± 0.25 respectively, $p = 0.19$). Subtraction of the basal level of PMN CD11b-expression from the level that was reached after *in vitro* LTB_4 up-regulation revealed that the absolute LTB_4-induced increase in PMN CD11b-surface molecules was significantly lower in psoriatic patients compared to healthy volunteers (6.26 ± 1.31 and 11.10 ± 0.69 respectively, $p = 0.001$).

No differences were observed between patients with stable and unstable psoriasis with respect to basal levels of PMN CD11b-expression, and LTB_4-stimulated PMN CD11b expression. However, when comparing patients with unstable psoriasis to healthy volunteers, the differences were more pronounced than they were between the overall group of patients and healthy volunteers. Both unstimulated and *in vitro* LTB_4-stimulated PMN CD11b-expressions were far lower in patients with unstable psoriasis ($p = 0.02$ and $p < 0.001$ respectively).

In order to assess whether the number of CD11b-molecules present on unstimulated blood-PMN is of relevance to the total number of these molecules present after *in vitro* up-regulation with LTB_4, the correlation between these two parameters was calculated in the psoriatic patients and healthy volunteers *(Fig. 3)*. There is a clear correlation between unstimulated and LTB_4-stimulated CD11b-expression in both groups ($p = < 0.001$).

Discussion

The present study showed a tendency to decreased expression of PMN CD11b in psoriatic patients compared to healthy

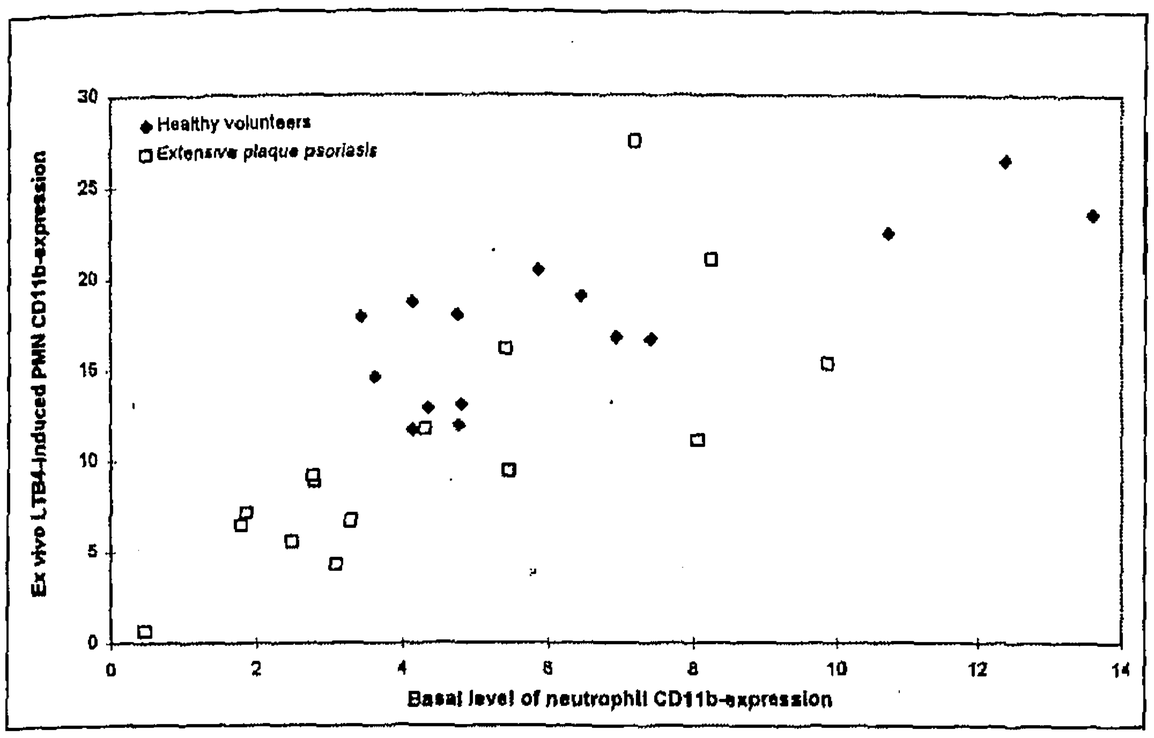

Figure 3. *Correlation between the number of CD11b molecules present on unstimulated peripheral blood PMN and the increased number that is present on these PMN after* in vitro *stimulation with LTB_4.*

considerably. However, in contrast to the difference with respect to absolute values, the relative CD11b up-regulation was virtually identical in patients and healthy subjects.
Experimental variability of flow cytometrical CD11b assessment proved to be below 10% comparing the triplicate measurements. Therefore, the present investigation reconfirms the high experimental reproducibility of the methods used.
In patients and healthy volunteers, the CD11b-levels on peripheral blood PMN following stimulation with LTB_4 correlate with the basal levels of CD11b, as illustrated in *Figure 3*. Therefore, unstimulated PMN CD11b expression determines LTB_4-induced PMN CD11b expression. The correlation between unstimulated and stimulated CD11b-levels in psoriatic patients and healthy controls seems to be comparable since there is no difference in relative CD11b up-regulation between both groups. This provides a strong indication that the signalling from LTB_4-receptor binding up to CD11b expression on peripheral blood PMN is essentially normal in psoriasis.
The tendency to decreased basal expression of CD11b and the significantly decreased LTB_4-induced psoriatic PMN CD11b expression compared to normal PMN indicates a decreased number of CD11b-hemireceptors on psoriatic PMN. The decreased expression of CD11b on peripheral blood PMN, further decreasing in unstable psoriasis, is in sharp contrast with the *in vitro* chemotaxis and protease activity which increases in unstable psoriasis [8].
Three explanations for the decreased CD11b expression of circulating psoriatic PMN may be hypothesized: (1) compartmentalisation of PMN subpopulations; (2) habituation to increased levels of leukotriene B_4; (3) active down-modulation of CD11b-levels on psoriatic peripheral blood PMN.
The hypothesis of compartmentalisation of circulating blood PMN is based on PMN subset selection. It is probable that the PMN-subpopulation with the highest CD11b expression is most likely to be recruited to invade the inflammed psoriatic skin, whereas PMN with a more modest density of CD11b remain in the blood circulation. Indeed, patients with unstable psoriasis proved to have an even lower CD11b expression on peripheral blood PMN than the overall group of psoriatic patients. In active psoriatic lesions, PMN-influx has been reported in 78% of patients and in chronic plaque lesions, PMN-influx proved to occur in 41% of patients [27].

An alternative explanation is habituation of peripheral blood PMN to increased LTB_4-levels present in psoriatic skin. This hypothesis is supported by the observation that in psoriatic uninvolved skin a decreased accumulation of PMN occurs following a standardized stimulus with LTB_4 [28, 29]. Repeated LTB_4 applications resulted in a decreased PMN accumulation as compared to the response following a single application [29]. However, in view of the fact that the relative CD11b up-regulation by LTB_4 in psoriatics proved to be essentially normal, this hypothesis is not supported by the observations in the present study.
Active down-modulation of CD11b is another possible mechanism that could explain the decreased PMN CD11b expression in psoriatic patients. It may be possible that such a defence mechanism exists in order to prevent massive cutaneous damage due to the abundant skin presence of PMN. To the best of our knowledge there are no known active down-modulators of CD11b. However, integrin α-units, like CD11b need divalent cations (calcium or magnesium) for their adhesive functions, and receptor function can be rapidly modulated through phosphorylation reactions [9]. It might well be possible that CD11b can be down-modulated on a functional level by changes in cation-concentrations and phosphorylase activity.
Since LTB_4-induced signalling in psoriatic PMN is essentially normal, the question arises as to what extent LTB_4 is relevant to the CD11b up-regulation of psoriatic PMN *in vivo*. The role of LTB_4 in psoriasis has been challenged further by the modest effects of 5-lipoxygenase inhibitors in the treatment of psoriasis [30-33].
The decreased CD11b expression on peripheral blood PMN in psoriasis remains an intriguing finding, and further studies should be aimed at mediators and factors involved in PMN compartmentalisation in psoriasis, and at mechanisms involved in the *in vivo* regulation of CD11b in psoriatic patients. ■

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